IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of	)	
	)	
Magnus Nilsson *et al.*	)	Confirmation No: 2591
	)	
Serial No. 10/584930	)	Examiner: to be assigned
	)	
Filing Date: July 5, 2006	)	Art Unit: to be assigned

For: CYSTEINE PROTEASE INHIBITORS

DECLARATION UNDER 37 CER 1.132

The undersigned hereby declares and states as follows:

that the undersigned is an inventor of the above identified application (the application);

that the undersigned is an employee of the assignee of the above identified application, with the title Principal Scientist, Medicinal Chemistry. Furthermore, the undersigned is currently, and at all material times has been, Project Director of the assignee company's cathepsin K program during which the subject invention was conceived and reduced to practice. The undersigned has a PhD in organic chemistry, is co-author in respect of several academic papers on the topic of cathepsin K mechanism and assay, and regularly presents on cathepsin K inhibition at international conferences;

that the data in the table bridging pages 102 and 103 in the above identified application was believed true, accurate and correct at the time the application was filed and formed the basis for specific statements on page 2, lines 7-8 and page 103, lines 2-3, but the data in the table is now not considered representative of results from repeated testing;

that repeated testing confirms the compounds according to the present invention demonstrate on average a potency of 3.07-fold that of their comparators rather than a 10-fold increase initially believed by applicants, based upon a single data pair in the table bridging pages 102 and 103 in the application;

that under applicants' supervision, direction and/or control, the following experimentation was conducted:

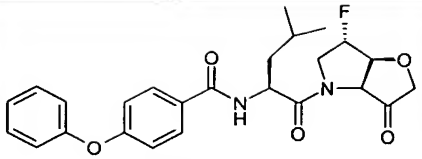
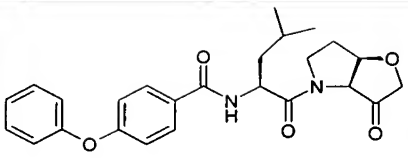
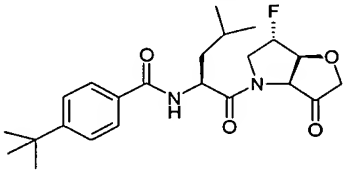
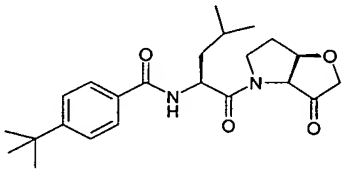
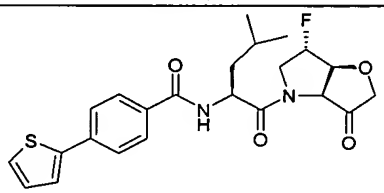
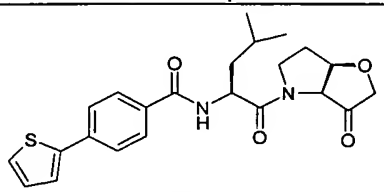
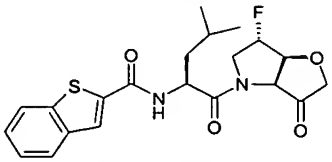
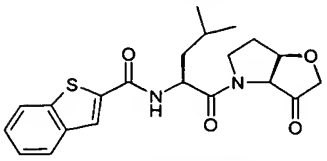
D4 - SUPPLEMENTARY EXPERIMENTAL DATA

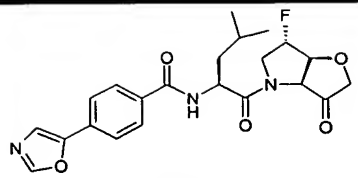
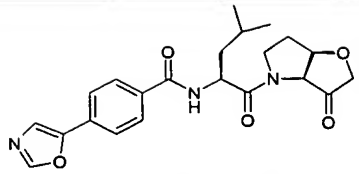
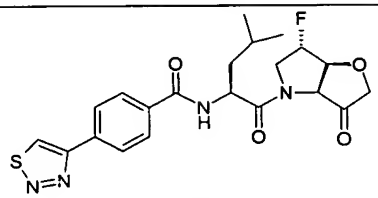
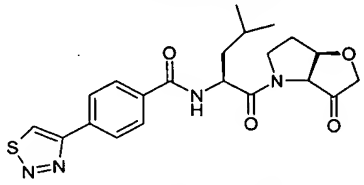
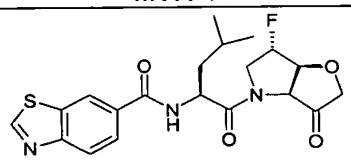
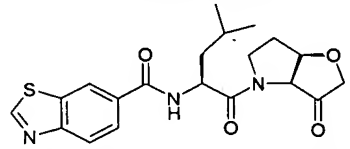
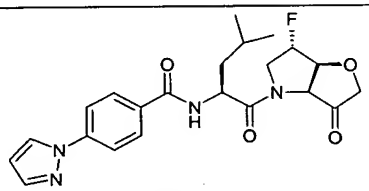
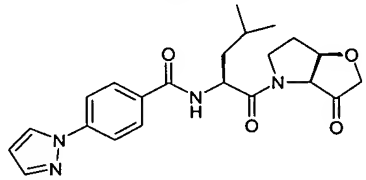
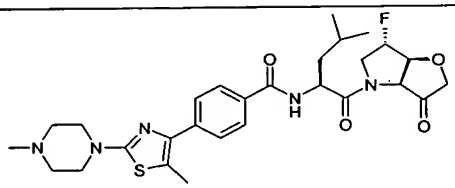
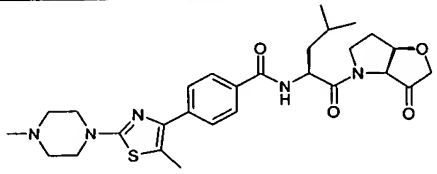
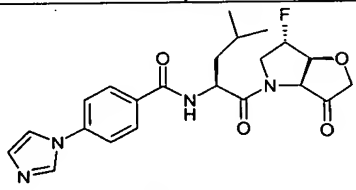
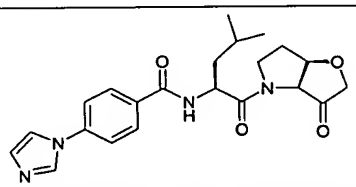
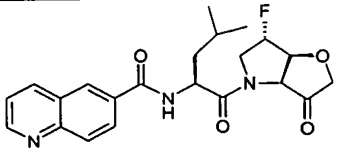
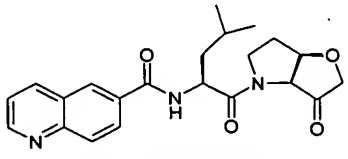
1. TEST COMPOUNDS

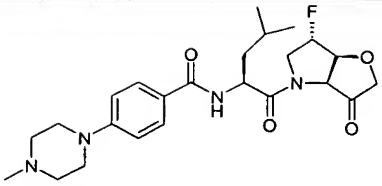
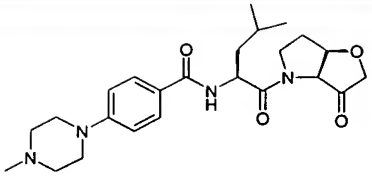
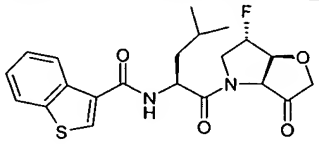
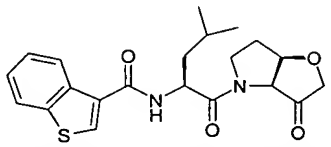
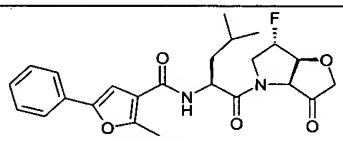
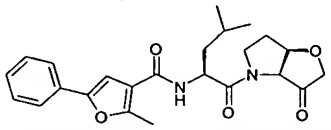
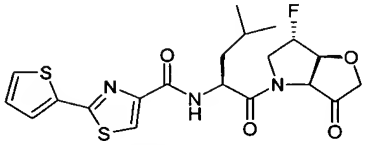
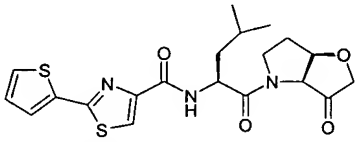
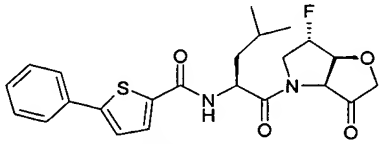
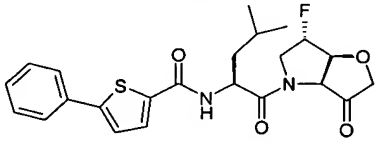
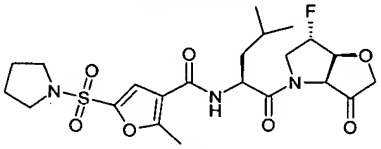
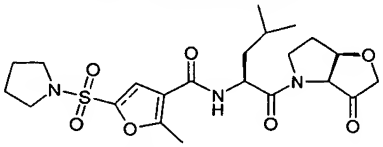
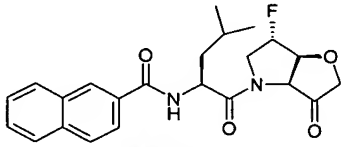
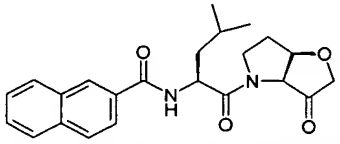
1.1 Comparative Pairs

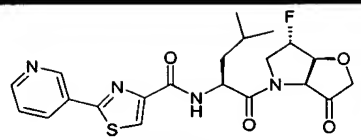
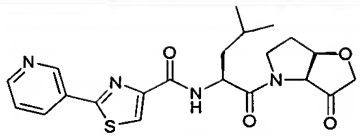
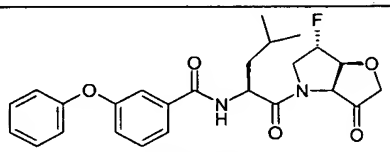
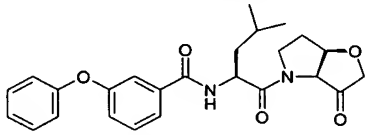
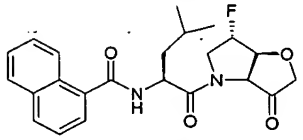
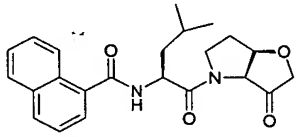
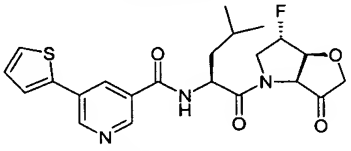
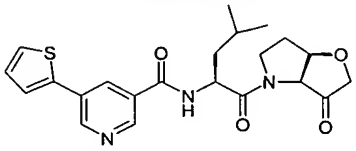
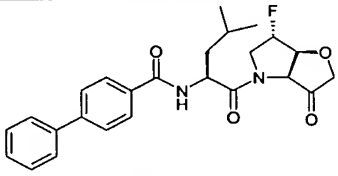
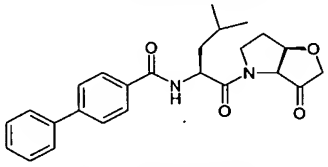
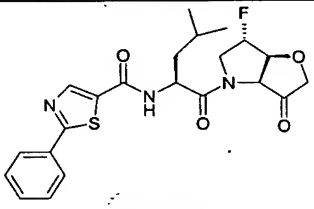
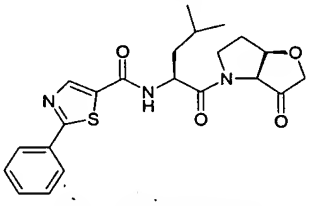
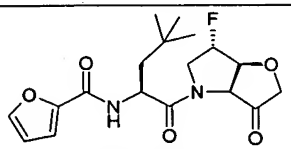
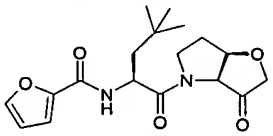
In order to demonstrate that the advantage associated with the compounds of the invention applies to a diverse range of chemical structures, a number of pairs of compounds were prepared for testing.

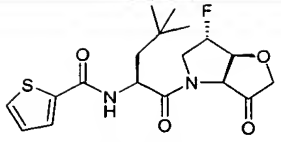
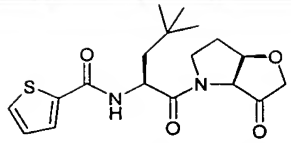
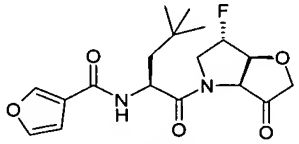
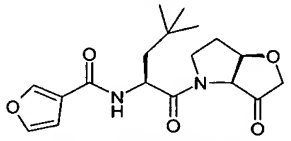
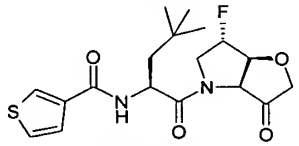
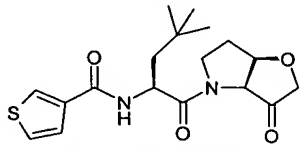
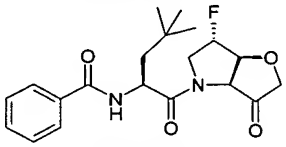
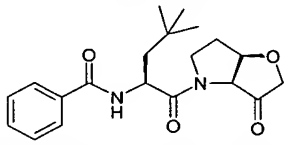
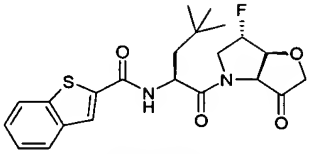
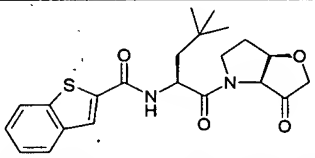
1.1.1 Compound Structures

Comparative Pair	Halogenated compound	Comparator compound
A	 Example 6	 D1 - Example 10
B	 MVR-1	 D1 - Example 9
C	 MVR-2	 D1 - Example 17
D	 Example 8.11	 D1 - Example 65

Comparative Pair	Halogenated compound	Comparator compound
E	 MVR-3	 D1 - Example 18
F	 MVR-4	 D1 - Example 22
G	 MVR-5	 D1 - Page 34 line 25 (+ page 28 line 27)
H	 MVR-6	 D1 - Page 35 line 17 (+ page 28 line 27)
I	 Example 9	
J	 MVR-7	 D1 - Page 35 line 5 (+ page 28 line 27)
K	 MVR-8	 D1 - Example 21

Comparative Pair	Halogenated compound	Comparator compound
L	 Page 14, left column, line 2	
M	 MVR-9	 D1 - Page 34 line 22 (+ page 28 line 27)
N	 MVR-10	 D1 - Page 36 line 7 (+ page 28 line 27)
O	 MVR-11	 D1 - Page 36 line 16 (+ page 28 line 27)
P	 MVR-12	 D1 - Example 20
Q	 MVR-13	 D1 - Page 36 line 19 (+ page 28 line 27)
R	 MVR-14	 D1 - Page 34 line 10 (+ page 28 line 27)

Comparative Pair	Halogenated compound	Comparator compound
S	 MVR-15	 D1 - Example 24
T	 MVR-16	 D1 - Example 11
U	 MVR-17	 D1 - Example 70
V	 MVR-18	 D1 - Example 23
W	 MVR-19	 D1 - Example 8
X	 MVR-20	 D1 - Example 19
Y	 MVR-21	 D1 - Page 88 line 20 (+ page 78 line 27)

Comparative Pair	Halogenated compound	Comparator compound
Z	 MVR-22	 D1 - Page 88 line 27 (+ page 78 line 27)
AA	 MVR-23	 D1 - Example 57
AB	 MVR-24	 D1 - Example 63
AC	 MVR-25	 D1 - Page 89 line 12 (+ page 78 line 27)
AD	 MVR-26	 D1 - Page 89 line 10 (+ page 78 line 27)

1.1.2 Synthesis

Comparator Compounds

The P1 building block for the comparator compound set was prepared and coupled to Murphy's linker as described in Example 1 of D1 (WO02/057270) in conjunction with pages 124-127. P2 and P3 units (which are commercially available, generally as an Fmoc protected species or are readily convertible to the Fmoc species) were coupled and the sequences cleaved as described on pages 124-127.

For the two compounds which had not been individualised in D1 (i.e. those in comparative pairs I and L), the preparation of the 4-[2-(4-methyl-piperazin-1-yl)-thiazol-4-yl] benzoic acid P3

building block is shown on page 69 of WO05/066180. The preparation of the 4-[N-methyl-piperazin-1-yl]benzoic acid (hydrochloride salt) building block is shown on pages 13 and 14 of WO01/58886 or can be prepared by Buchwald chemistry (*J.Org. Chem.* 2000 65:1144), as recited at page 34 of WO05/066180.

The NMR and MS readouts were consistent with those reported in Example 1 or the respective end-product Examples of D1. The MS readouts of non-exemplified compounds were consistent with predictions.

Compounds of the Invention

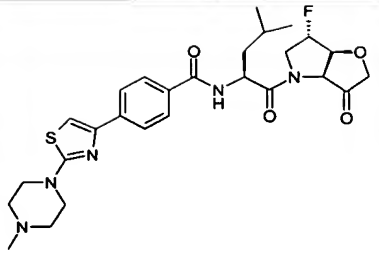
The corresponding halogenated compounds MVR-1 to MVR-26 were prepared on solid phase and cleaved as described in Example 8 of WO05/066180 using the same commercially available Fmoc protected P2 and P3 building blocks as above. The MS readouts were consistent with predictions.

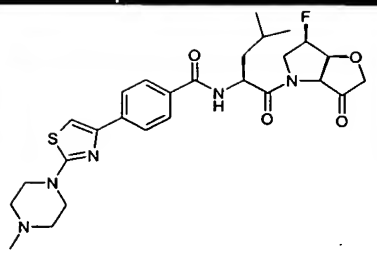
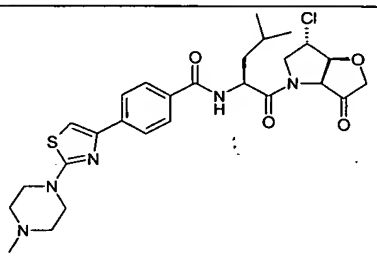
The preparation of the remaining compounds, i.e. Examples 6, 8.11 and 9 are shown in the respective examples of WO05/066180. The 4-[N-methylpiperazin-1-yl]benzoic acid -P3 building block is described above.

1.2 Alternative Stereochemistry/Halogen

To demonstrate that the benefits for down-fluoro compounds extend to other halogen configurations an exemplary up-fluoro enantiomer and down-chloro enantiomer were prepared.

1.2.1 Compound Structures

Compound Structure	Compound Description
	Example 8.19 ('Down-fluoro')

Compound Structure	Compound Description
	'Up-fluoro' stereoisomer
	'Down-chloro' halogen analogue

1.2.2 Synthesis

The compound of Example 8.19 above was prepared by solution phase chemistry using the general procedure as described in Example 9 of WO05/66180, but employing the appropriate P3 building block described at page 69 of WO05/66180.

The P1 building block for the 'up-fluoro' stereoisomer was prepared by benzyl protecting compound 55 from Example 1 of WO05/66180, oxidizing and ketal protecting the ketone as shown in Example 9 and treating with the fluorinating agent Deoxyfluor™ in DCM. The benzyl N-protecting group was removed, and the P2 and P3 units coupled and keto function deprotected in solution phase as shown in Example 9 of WO05/66180 to yield the final product.

The P1 building block for the 'down-chloro' halogen analogue was prepared by protecting the hydroxyl group of compound 10 of WO05/066180 as the hemiacetal, analogously to Example 9. The benzyl and CBz protecting groups were then removed, the N function re-protected with CBz and the free hydroxyl protected as the mesylate. The chlorine was introduced with lithium chloride and the CBz removed. Coupling of the P2 and P3 units and deprotection to the keto was done in solution phase as shown in Example 9 of WO05/66180 to yield the final product.

2. BIOLOGICAL ASSAYS

2.1 Assay Method

Compounds were tested for their ability to inhibit cathepsin K activity according to the procedure described on pages 101-102 of the original application.

2.2 Results

2.2.1 Comparative Pairs

Comparison Pair	Compound of the Invention Mean Ki (nM)	SD	n	Comparator Compound Mean Ki (nM)	SD	n	Ratio of means	Probability of difference	Probability >2 fold difference
A	18.91	6.96	8	44.78	19.78	9	2.37	0.998	0.780
B	3.83	1.92	7	8.20	4.40	6	2.14	0.970	0.615
C	8.24	1.77	8	22.86	11.96	7	2.77	0.991	0.806
D	6.96	2.39	9	10.60	2.84	7	1.52	0.991	0.054
E	18.13	7.20	8	59.57	28.92	7	3.29	0.996	0.972
F	27.00	5.58	11	109.86	62.17	7	4.07	0.994	0.988
G	20.15	11.03	8	77.00	45.73	7	3.82	0.992	0.944
H	24.50	7.89	8	122.71	69.39	7	5.01	0.995	0.994
I	3.13	0.93	17	8.43	4.81	7	2.69	0.987	0.937
J	37.18	12.06	11	93.17	16.87	6	2.51	1.000	0.960
K	48.27	28.17	11	153.43	84.81	7	3.18	0.992	0.939
L	2.76	2.26	8	6.17	1.74	6	2.24	0.996	0.842
M	49.86	12.47	7	187.71	94.93	7	3.77	0.996	0.975
N	188.57	63.62	7	1140.00	689.57	7	6.05	0.995	0.994
O	123.86	51.80	7	621.43	363.43	7	5.02	0.995	0.991
P	8.06	2.70	7	9.64	5.38	7	1.20	0.748	0.026
Q	255.71	74.58	7	1104.29	447.13	7	4.32	0.999	0.999
R	16.97	8.62	7	24.78	14.55	11	1.46	0.914	0.120
S	254.29	65.28	7	778.57	336.42	7	3.06	0.997	0.983
T	137.43	44.24	7	707.14	369.45	7	5.15	0.997	0.999
U	1271.43	652.90	7	1824.29	1284.70	7	1.43	0.832	0.094
V	682.86	358.46	7	2285.71	1440.40	7	3.35	0.987	0.960
W	18.57	7.91	7	75.75	42.59	8	4.08	0.997	0.980
X	117.57	39.17	7	305.71	127.26	7	2.60	0.996	0.847
Y	55.00	2.61	6	166.67	16.33	6	3.03	1.000	1.000
Z	17.17	2.86	6	50.60	3.51	5	2.95	1.000	1.000
AA	69.80	19.20	5	180.00	31.62	6	2.58	1.000	0.968
AB	14.33	1.51	6	34.83	3.97	6	2.43	1.000	0.995
AB	35.33	1.87	12	82.83	5.85	6	2.34	1.000	1.000
AD	4.35	0.54	6	8.00	1.00	6	1.84	1.000	0.148

2.2.2 Alternative Stereochemistry/Halogen

Compound Description	Ki (nM)
Example 8.19 ('Down-fluoro')	1.4
'Up-fluoro' stereoisomer	1.3
'Down-chloro' halogen analogue	1.0

3. CONCLUSIONS

3.1 Comparative Pairs

It is apparent that there is a degree of inherent variability between tests. To ensure that a statistically relevant data set was produced, each individual compound in the comparative pairs was tested at least 6 times. Only one deviant value has been excluded from the results (in respect of MVR-9), which was over an order of magnitude away from other tests of the same compound.

- In every case the compound of the invention was found to be more potent than the corresponding compound which is absent the inventive feature.
- In 25 out of the 30 pairs the compound of the invention was found to be at least twice as potent.
- In almost half of the comparative pairs the average Ki value for the inventive compound was found to be at least three times more potent.
- The mean ratio for all the pairs indicates that the compound of the invention was on average 3.07 times more potent, with a standard deviation of 1.21.

Treating the effect of halogenation at this position of the molecule as being independent of other structural changes, a Z-test can be used to assess the statistical significance of the findings.

- Probability of the real increase in potency being at least 2-fold is 99.99%
- Probability of the real increase in potency being at least 3-fold is 75.5%

Consistent potency increases of this magnitude have a tremendous practical significance in that the necessary dose and hence potential toxicity of the compound is at least halved for a given efficacy. This is particularly important for drugs in indications such as osteoporosis where

lifelong compliance is required (i.e. the drug may be taken for decades, meaning that cumulative toxicity is a real issue). Additionally, cost of goods for a chronic treatment is an important factor, which benefits from substantially increased potency.

3.2 Alternative Stereochemistry/Halogen

Each of the comparative pairs incorporates a downwardly facing fluorine atom. Nevertheless, direct comparison of the 'down-fluoro' compound to its 'up-fluoro' and 'down-chloro' analogues indicates that the general effect may be considered representative of other stereochemical configurations and other halogen atoms.

that the above information shows the compounds according to the present invention demonstrate on average a potency of 3.07-fold that of their comparators;

that the table in section 2.2.1, in addition to the experimental data relating to the compound of the invention (columns 2-4) and their unhalogenated comparators (columns 5-7), also includes the ratio of the mean Ki (column 8), the probability of the compounds not being equivalent (column 9) and the probability of the inventive compound being at least twice as potent as the comparator (column 10). The ratio of the means value is simply the mean Ki for the comparator (column 5) divided by the mean Ki for the inventive compound (column 2), it provides an indication of the potency of each individual inventive compound relative to its comparator. The value is greater than 1 in every case, as the mean Ki for each inventive compound is always lower (i.e. more potent) than the mean Ki of the comparators. The mean of the ratios (i.e. the mean average of the values in column 8), which is found to be 3.07, provides the average relative potency across all comparative pairs. Consequently, the compounds of the invention demonstrate on average 3.07 times the potency of their unhalogenated comparators;

and that in the data presented above, one deviant value was excluded from the statistical analysis, specifically in respect of MVR-4 (the excluded value of 560 nM being a clear outlier in light of the mean and standard deviation of the remaining 10 determinations being 27 nM and 5.6 respectively);

All statements made herein of my own knowledge are true and that all statements made on information and believe are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Urszula Grabowska

29th October 2008

Date